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草莓中黄酮类物质的测定以及与抗氧化活性之间的相互关系

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摘要: 报道草莓中黄酮类物质含量的测定方法, 并比较了总抗氧化成分与抗氧化活性 (DPPH, FRAP) 之间的相互关系。黄酮类物质的水解随着 HCl 浓度和水解时间的不同发生较大的差异, 结果表明, 总多酚类物质和总黄酮类物质之间有很高的相关关系, 总多酚类物质和总黄酮类物质对 DPPH 有很高的相关关系, 而且总多酚类物质和总黄酮类物质对 FRAP 的相关关系各为 $r = 0.958$, $P < 0.05$ 和 $r = 0.936$, $P < 0.05$, 从结果可以推测: 草莓体现出很强的抗氧化活性, 这与草莓中的总多酚类物质成分密不可分。

关键词: 草莓; 水解浓度; 水解时间; 总多酚类物质; 总黄酮类物质; DPPH; FRAP
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Quantification of Flavonoid Compounds from Strawberry (*Fragaria ananassa*) and Correlation Effects with Antioxidant Activities

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Abstract: The amounts of flavonoid (myricetin, quercetin and kaempferol), antioxidant contents (total phenolic and total flavonoid) and antioxidant activities (DPPH and FRAP) were analyzed in strawberry. Quantitative analysis of conjugated flavonoid contents were obtained after different hydrolysis concentration and reaction period. The highest yields of myricetin and quercetin were found with 1.0M HCl and a reaction period of 0.5 hr, however, for kaempferol was 1.0M HCl and a reaction period of 1hr, respectively. In the study, we also estimated the correlation coefficient of TPC, TFC, DPPH and FRAP. In addition, a positive correlation was observed ($r = 0.980$, $P < 0.05$) between TPC and TFC, TPC and TFC are correlated to the DPPH activity ($r = 0.913$, $P < 0.05$ and $r = 0.899$, $P < 0.05$, respectively), and to the FRAP activity ($r = 0.958$, $P < 0.05$ and $r = 0.936$, $P < 0.05$, respectively), respectively. These results pointed out that strawberry generally possesses a high level of antioxidant activities, which could be linked to the levels of phenolic compounds in the fruit.

Key words: Strawberry; Hydrolysis concentration; Reaction period; Antioxidant contents; Antioxidant activities; Correlation coefficient

Strawberry fruits, with it special sweet, sour taste and exquisite flavor, especially have previously been shown to contain antioxidant compounds such as flavonoid (myricetin, quercetin and kaempferol) and phenolic acids, which provide protection against harmful free radicals (Vinson *et al.*, 2001, Cai *et al.*, 2004, Jose *et al.*, 2007). Flavonoids have been detected in

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many studies with plants, measured by after acid hydrolysis, because most flavonoids founded in plants are conjugated to sugars as glycosides, hydrolysis of all glycosides to aglycones offers a practical method for the quantitative determination of flavonoids in plant (Hertog and Peter, 1992; Alan *et al*., 1997).

To the best of the author's knowledge, the flavonoids aglycones in strawberry have not yet been estimated according different experiment design of hydrolysis concentration and reaction period. Furthermore the correlation among antioxidant contents such as total phenolic content (TPC), total flavonoid content (TFC), and free radical scavenging activity of DPPH, FRAP also have not been reported. The objectives of this study were 1) to optimize the methods of extraction and hydrolysis of flavonoids conditions and 2) to determine DPPH and FRAP activity to estimate antioxidant activities and their correlations with TPC and TFC in strawberry fruit.

Materials and Methods

Strawberry (*Fragaria ananassa* Duch.) was purchased from the local market in Dae-gu Korea.

Extraction and hydrolysis fruit for flavonoid aglycones analysis were obtained by different reaction and acid concentration. And the treated sample was dissolved with methanol and then filtered through a 0.45 μm membrane filter for HPLC analysis.

The total phenolic content (TPC) and DPPH was determined according Cai *et al*. (2004), measurement of total flavonoid content (TFC) and FRAP was determined according to Faria *et al*. (2005) with some modifications.

Results and Discussion

We estimated the optimum extraction and hydrolysis conditions for quantifying the fruit of strawberry, because the completeness of hydrolysis largely depended on the type of glycosides. Four hydrochloric acid concentrations were tested (0.5, 1.0, 1.5 and 2.0 mol/L), and the reaction period was varied (0.5, 1 and 2 hrs) in the procedure described under extraction and hydrolysis. The influence of acid concentration and reaction period corresponding aglycone yield is presented in table 1.

In our study, RP-HPLC coupled with UV-VIS Detector was employed to separate, identify and quantify favonoid aglycones in strawberry fruit (Fig. 1). In this study, the highest yields of myricetin and quercetin were found with 1.0 mol/L HCl and a reaction period of 0.5 h, however, for kaempferol was 1.0 mol/L HCl and a reaction period of 1 h, respectively. After 2 hrs with 2 mol/L HCl concentration, a loss of up to 50.3% and 41.7% of myricetin and quricetin was observed compare to the optimum conditions, respectively (Table 1). These findings supported those of the study by Alan *et al*. (1997) who reported that cooking will fall in quercetin and myrecitin content, boiling produced even bigger reduction in tomatoes and onions. All these results indicated that prolonged or shortened extraction and hydrolysis showed a decrease the flavonol contents in strawberry.

Table 1 Quantification data for optimization of hydrolysis conditions in strawberry fruit (100 mg g^{-1} fresh weight)

HCl Conc. (mol/L)	time (h)	Myricetin	Quercetin	Kaempferol	Total
0.5	0.5	4.48	5.95	N.D[a]	10.43
	1	4.63	6.21	1.48	12.32
	2	4.13	6.07	1.32	11.52
1.0	0.5	5.09	6.60	1.45	13.14
	1	4.69	5.89	1.92	12.5
	2	3.31	5.34	1.12	9.77
1.5	0.5	4.89	6.12	1.55	12.56
	1	3.27	5.38	1.75	10.4
	2	2.86	3.66	1.66	8.18
2.0	0.5	3.88	5.02	1.77	10.67
	1	3.06	4.23	1.67	8.96
	2	2.56	2.75	N.D[a]	5.31

[a] N.D., Not detected.

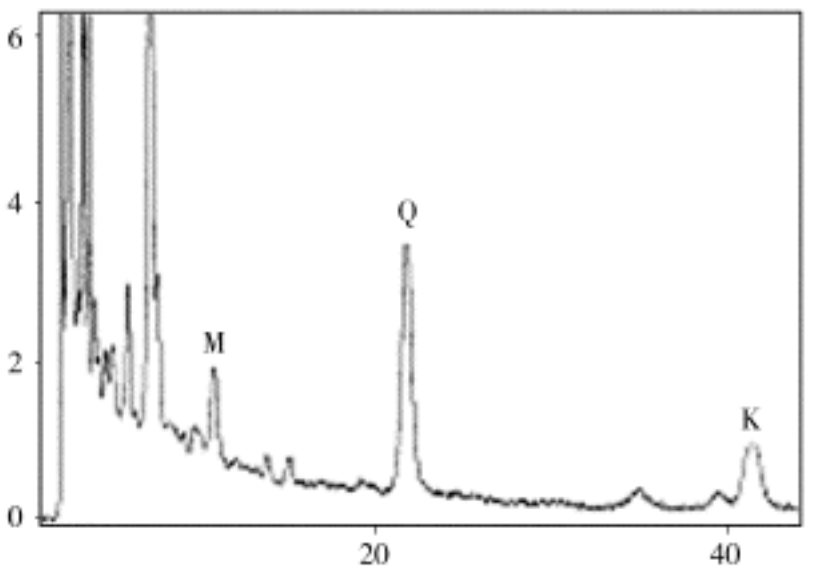

Fig. 1 Chromatograms of flavonoid aglycones, strawberry extracts and hydrolysis with 1.0 mol/L HCl and a reaction period of 1 h
M: myricetin; Q: quercetin; K: kaempferol

The TPC and TFC in the strawberry fruits in methanol extraction showed the higher value of TPC (4575.8 ± 80.8 mg GAE/100 g F.W.) and TFC (430.7 ± 28.5 mg QE/100 g F.W.) compare to dichloromethane extraction of TPC (41.4 ± 5.3 mg GAE/100 g F.W.) and TFC (3.8 ± 2.7 mg QE/100 g F.W.), respectively. Our result was agreement with those reported by Kriengsak *et al*. (2006), who showed that TPC in methanol extraction is very higher compare to dichloromethane extraction. All these results indicate that methanol extraction is a better extraction solvent for the extraction of both TPC and TFC.

The methanol extraction exhibited a concentration-dependent

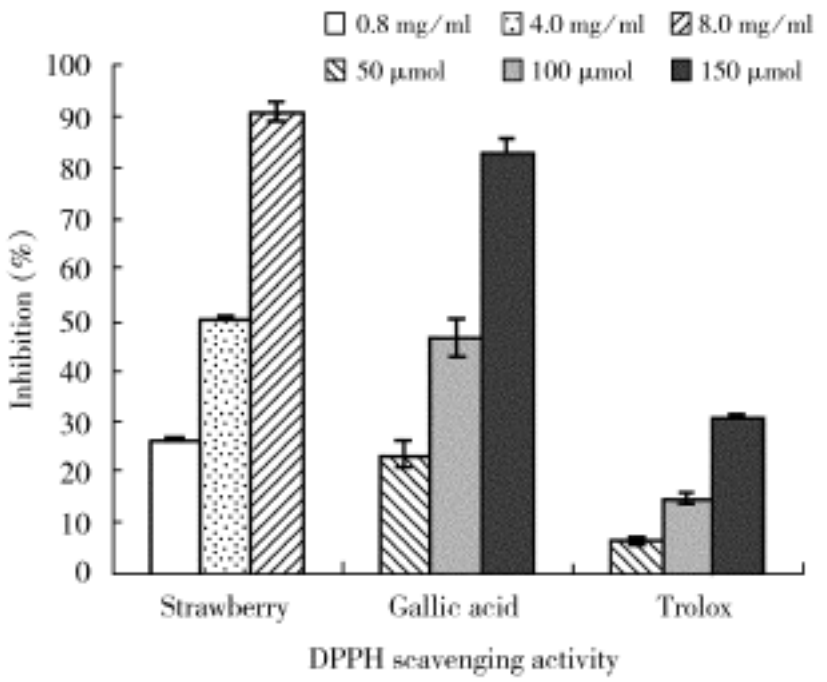

Fig . 2　Free radical scavenging activity (DPPH) of strawberry
means ± SD, n = 3 . Bars represent standard error

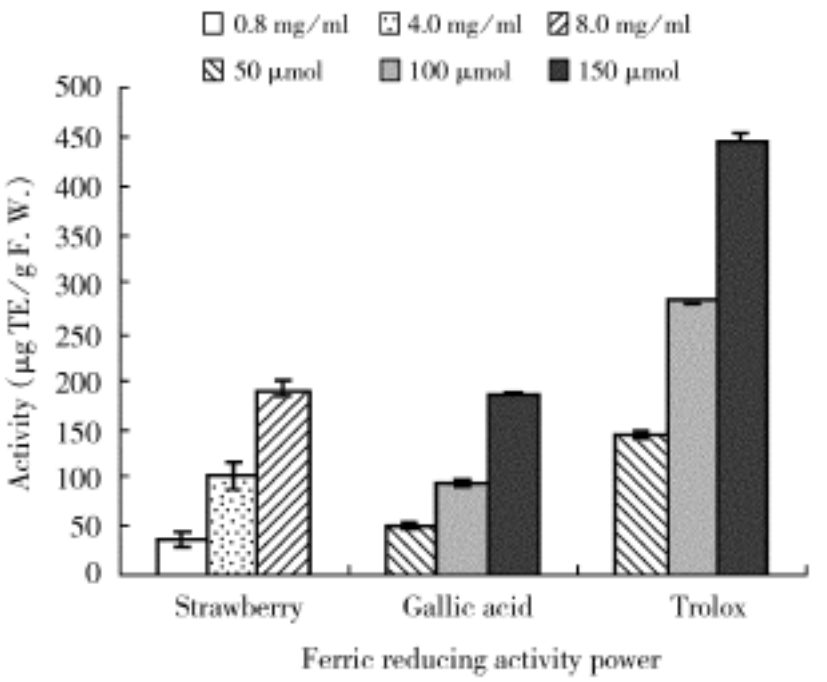

Fig . 3　Ferric reducing antioxidant power (FRAP) of strawberry
means ± SD, n = 3 . Bars represent standard error

DPPH radical scavenging activity, which was higher than that of the positive control, trolox, the scavenging effect increased with the increasing concentrations from 0.8 - 8.0 mg/ml, it was found to be over 90.8% in 8.0 mg/ml concentration, which is significantly higher than that of gallic acid (82.9%) and trolox (30.4%) at the same concentration of 150 μmol (Figure 2). These results were agreement with Jose *et al.* (2007) who indicated that native cultivated strawberries (*F. vesca*) were 2.5 times more active than in the TEAC assay (Trolox equivalent antioxidant capacity).

Figure 3 clearly showed that the FRAP of methanol extract of strawberry had reducing power of 191.5 ± 7.2 TE μmol/mg extract. This was slight higher than that of ascorbic acid (184.5 ± 5.6 TE μmol/mg), but lower than that of gallic acid (447.7 ± 5.9 TE μmol/mg) within a concentration of 150 μm. According to its high value, it could be considered that compound was good electron donors and could terminate oxidation chain reactions by reducing the oxidized intermediates into the stable form.

Recently studies have suggested that the antioxidant contents and antioxidant free radical scavenging activities have the positive effect (Jose *et al.*, 2007, Kriengsak *et al.*, 2006). In our study with the strawberry fruit, a positive correlation was observed ($r = 0.980$, $P < 0.05$) between TPC and TFC in methanol extraction, however, there was no significant correlation in dichloromethane extraction (data not shown). Furthermore, TPC and TFC are correlated to the DPPH activity ($r = 0.913$, $P < 0.05$ and $r = 0.899$, $P < 0.05$, respectively) and to the FRAP activity ($r = 0.958$, $P < 0.05$ and $r = 0.936$, $P < 0.05$, respectively) was observed. In addition, a positive correlation was also observed ($r = 0.947$, $P < 0.05$) between DPPH and FRAP. Our results indicated that TPC showed the highest correlation with TFC. It could be explain that phenolic and flavonoid compounds, which are known as hydrophilic antioxidants, are secondary metabolites that are most abundant in fruits, which positively correlated with the oxygen radical absorbance capacity (ORAC). In addition, most techniques used for determining antioxidant activity, showed high correlation with TPC and TFC in different free radical scavenging activities. All these results were agreement with Vinson *et al.* (2001) who reported that strawberry generally possesses a high level of antioxidant activity, which could be linked to the levels of phenolic compounds in the fruit. Furthermore, our results were supported by Gil *et al.* (2002), who found high correlation between antioxidant activities and total phenolic content in nectarines, peaches and plums, and they demonstrated that there was no correlation between ascorbic acid and antioxidant activity as determined by DPPH or FRAP assays.

Strawberry fruits contain a group of natural antioxidants that could have not only a high antioxidant activity but also a good combination or mixture of antioxidants, the supplementation of these natural antioxidants through a balanced diet containing enough fruits could be much more effective and also economical than the supplementation of synthetic preservatives.

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